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R Vigneshwar
M.V.Sc Scholar, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Arivuchelvan Arivalagan
Professor and Head, Department of Animal Husbandry Statistics and Computer Applications, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Mekala Palanivel
Assistant Professor, Ethno Veterinary Herbal Research Centre for Poultry, Namakkal, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Corresponding Author:
R Vigneshwar
M.V.Sc Scholar, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Thyrogenic, hypolipidemic and antioxidant effects of *Bacopa monnieri* (Brahmi) on experimental hypothyroidism in rats

R Vigneshwar, Arivuchelvan Arivalagan and Mekala Palanivel

Abstract
Bacopa monnieri commonly known as Brahmi is one of the commonly available and well known herbs in India. It has been used as a brain tonic and recommended for treating poor cognition and lack of concentration in humans. The present study was undertaken to assess the effects of Brahmi on experimentally induced hypothyroidism in male Wistar rats, which is less known. Hypothyroid rats were treated with 200 mg per kg of Brahmi and levothyroxine has been used as a standard replacement drug. Plasma levels of thyroid hormones (T_3, T_4 & TSH), lipid profile and liver anti-oxidants (catalase, superoxide dismutase, reduced glutathione and lipid peroxidation levels) were determined. Histological study of the thyroid glands was carried out. The results thus obtained suggest that Brahmi ameliorated hypothyroidism as evidenced by reversal of various biochemical changes as well histology of thyroid gland in rats. Thus the plant could be considered for therapeutic management of clinical conditions associated with hypothyroidism.

Keywords: Brahmi, hypothyroidism, wistar rat, levothyroxine, anti-oxidant

Introduction
Hypothyroidism is a common endocrine disorder resulting from deficiency of thyroid hormone or, more rarely, from their impaired activity at tissue level. Primary hypothyroidism is due to insufficient production of thyroid hormone by thyroid glands, secondary due to inadequate secretion of thyrotropin (TSH) from the pituitary gland and tertiary due to deficiency of thyrotropin releasing hormone (TRH) from the hypothalamus [1]. Untreated hypothyroidism leads to hypertension, dyslipidaemia, infertility, cardiomyopathy, anaemia and neuromuscular dysfunction [2].

Rat and mice are commonly used animal models for experimental thyroid disorders. Administration of thionamide compounds such as propylthiouracil, methimazole or thiourea can induce hypothyroidism of which propylthiouracil (PTU) is commonly used as an induction agent in experimental biology. It inhibits intra thyroidalsynthesis of thyroid hormones by interfering with thyroid peroxidase mediated iodide trapping, which leads to reduced concentrations of thyroxine (T_4) and triiodothyronine (T_3) in blood. In addition, PTU inhibits type-1 deiodinase which is responsible for the peripheral conversion of T_4 to T_3 [3].

Levothyroxine sodium is a common replacement drug used to treat hypothyroidism. In addition to its desirable effects, some side effects may be caused by levothyroxine which includes behavioral change, heat intolerance, tachycardia, irregular breathing, restlessness, insomnia, abdominal cramps, palpitation and weight loss [4]. Hence it is envisaged that evaluating an herbal alternative for treatment of hypothyroidism would improve the quality of life of pet animals.

Bacopa monnieri (BM), also known as Brahmi, water hyssop and *Bacopa monniera*, is a creeping perennial herb found in marshy habitats. The plant is endemic in Asia, but can also be found in parts of Africa, Australiaand the Southeastern states in the USA. Bacopa has long been used as a medicinal herb by Ayurvedic physicians and the practitioners of the traditional system of medicine of India. Bacopa was first documented in several ancient Ayurvedic texts including the Charaka Samhita and the Sushruta Samhita, where clear reference was made to its action on the central nervous system [5]. It has been used as a brain tonic and recommended for the management of anxiety, poor cognition, and lack of concentration [6].

Bacopa has also been used to treat some inflammatory conditions such as, asthma, bronchitis, dropsy, and rheumatism [7, 8]. However, less is known regarding the pharmacological actions of bacopa in hypothyroid patients. The existing literature suggested the thyrogenic action of BM in euthyroid mice [9].

However, an experimental animal model of hypothyroidism will serve as a better tool to study the ameliorative actions of BM in hypothyroidism and complications associated with it.
Taking the above points into consideration, this work has been carried out with the following objectives:

1) To induce hypothyroidism in rats and evaluate the thyroid stimulatory activity of *Bacopa monnieri*
2) To study the effects of *Bacopa monnieri* on complications related to hypothyroidism such as hyperlipidemia and oxidative stress in comparison with the standard replacement drug L-thyroxine.

Materials and Methods

Collection of plant material and extraction procedure

The plant *B. monnieri* leaves were collected from the herbal garden of Veterinary College and Research Institute, Namakkal. The leaves were shade dried and powdered using blender. The leaf powder was cold extracted with 70% ethanol and kept in rotatory shaker for 48 hrs. Then it was filtered with Whatmann filter paper No.1 and kept in an incubator at 37 ºC to obtain semi solid substance. The dried extract was weighed and stored at 4 ºC in an air tight container. The alcoholic extract was dissolved in water and administered through gastric intubation.

Phytochemical Screening

The extract was subjected to preliminary qualitative phytochemical analysis to test the presence of active phytocompounds such as tannins, saponins, terpenoids, steroids, flavonoids, phenol, alkaloids, glycosides and cardiac glycosides by standard procedures described previously [10].

Experimental Animals

Thirty healthy young male Wistarrats procured from the Department of Laboratory Animal Medicine, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram milk colony, Chennai-51 were used for this study. The rats were acclimatized for a period of 10 days prior to the start of experiment. Rats were reared in polypropylene cages under standard managemental conditions as per the CPCSEA guidelines and provided *ad libitum* feed and water throughout the experimental period of 8 weeks. All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee of Veterinary College and Research Institute, Namakkal-02.

Experimental Design

The rats were randomly divided into five groups of six rats each and subjected to the following treatment:

Group I: Normal control group: rats received distilled water

Group II: Hypothyroid group: Rats received PTU @ 15mg/kg body weight for 30 days + distilled water for last 30 days

Group III: Standard group: Rats received PTU @ 15mg/kg body weight for 30 days + Levothyroxine sodium @ 20µg/animal for last 30 days

Group IV: Test group: Rats received PTU @ 15mg/kg body weight for 30 days + 200 mg of BM for last 30 days

Group V: Herb control group: Rats received distilled water for 30 days + 200 mg of BM for last 30 days

Experimental Induction of Hypothyroidism

Hypothyroidism was induced in animals of group II to IV by administration of propylthiouracil (PTU) dissolved in distilled water through oral route at the dose rate of 15mg/kg body weight per day for 30 days. On 31st day, blood was collected from each rat from orbital sinus, centrifuged and plasma samples were subjected to Radio Immuno Assay (RIA) for the estimation of total T_3, T_4 and TSH for the confirmation of induction of hypothyroidism. Then the animals were treated as per the details given above in the experimental design.

Sample collection

At the end of the experiment, all the animals were fasted over night and sacrificed by CO_2 asphyxiation. Blood was collected in heparin coated vials, centrifuged and plasma was separated for hormonal and biochemical assays. Thyroid and liver were dissected out immediately, washed with phosphate buffered saline and dried with filter paper. Thyroid glands were fixed in 10 per cent neutral buffered formalin for histopathological investigation. Liver tissue homogenate was subjected to anti-oxidants assays.

Biochemical analysis

Plasma levels of T3, T4 and TSH were measured by Radio Immuno Assay (RIA) in Gamma counter by using commercial kits from Beckman Coulter according to the manufacturer's instructions. Plasma levels of total cholesterol, triglycerides and HDL cholesterol were measured by using commercial kits from Span Diagnostics according to the manufacturer's instructions. LDL-Cholesterol and VLDL-Cholesterol levels were calculated mathematically according to Friedewald's equations [11]. The anti-oxidant enzymes super oxide dismutase (SOD) catalase (CAT), total reduced glutathione (GSH) and lipid peroxidation (MDA) levels of liver tissues were measured spectrophotometrically according to previously reported methods [12, 15].

Histopathology

Thyroid glands were collected along with trachea and fixed in 10% formalin, then subjected to dehydration with descending grades of isopropyl alcohol and xylene, followed by paraffin embedding and sectioned at 5 µm thickness, stained with hematoxylin and eosin (H&E) and examined microscopically.

Statistical Analysis

All data are presented as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used to compare the means. Post-hoc analysis was done by Duncan's multiple range tests by using SPSS® 20.0 software package.

Results

Phytochemical Screening

The phytochemical characterization of ethanolic extract of *Bacopa monnieri* is given in table 1. The extract has shown positive for terpenoids, steroids, phenols, saponin, coumarins, tannins, flavonoids, cardiac glycosides and alkaloids and negative for quinones and glycosides. The results confirmed the presence of therapeutically potent compounds in the alcoholic extract of *Bacopa monnieri*.

Table 1: Preliminary Phytochemical Screening of ethanolic leaf extract of BM

Phytochemical compounds	Method	Result
Tannins	Ferric chloride test	+
Saponins	Foam test	+
Quinones	Sulfuric acid test	-
Terpenoids	Noller test	+
Steroids	Salkowski test	+
Flavonoids	Shinoda test	+
Glycosides	Fehling's test	-
Alkaloids	Dragendorff test	+
Cardiac glycosides	Keller Killani test	+
Phenol	Ferric chloride test	+
Coumarins	Sodium hydroxide test	+

+= positive; - = negative

Effect of BM on Plasma thyroid hormones level

The mean total T_3, T_4 and TSH level of all the groups (Group I to Group V) at the end of the experiment are presented in Table 2. A highly significant (P<0.01) reduction in total T_3 and T_4 level and highly significant increase in TSH level was observed in hypothyroid group (II) when compared to control group (I). There was no significant difference among the control group (I), levothyroxine treated group (III) and BM 200 mg treated group (IV). However, the herb control group (V) showed significant increase in T_3 and T_4 level and decrease in TSH level when compared to other groups.

Table 2: Effect of BM on Plasma thyroid hormones level

Parameters	Group I	Group II	Group III	Group IV	Group V
T_3 (nmol/L)*	$2.61^{b} \pm 0.24$	$0.20^{c} \pm 0.03$	$2.33^{b} \pm 0.38$	$2.58^{b} \pm 0.15$	$3.14^{a} \pm 0.22$
T_4 (nmol/L)*	$54.18^{b} \pm 2.47$	$15.08^{c} \pm 1.72$	$51.41^{b} \pm 1.75$	$54.27^{b} \pm 1.24$	$61.68^{a} \pm 1.74$
TSH (mIU/L)*	$0.34^{b} \pm 0.01$	$1.55^{a} \pm 0.12$	$0.36^{b} \pm 0.02$	$0.33^{b} \pm 0.01$	$0.28^{c} \pm 0.03$

*Overall means bearing different superscripts within row differ highly significantly (P< 0.01) Values were expressed as mean ± SEM for 6 rats in each group

Effect of BM on Plasma lipid profile

The mean lipid profile of all the groups (Group I to Group V) at the end of the experiment is presented in Figure 1. A highly significant (P<0.01) increase in plasma total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterollevel and significant (P<0.05) decrease in HDL cholesterol level was noticed in hypothyroid group when compared to control and treatment groups. All the other groups showed a significant reduction in plasma lipid profile compared to hypothyroid group and did not differ significantly from the control.

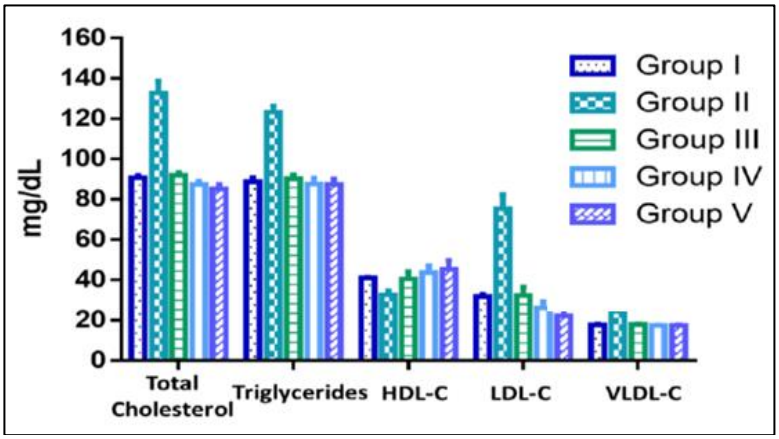


Fig 1: Effect of BM on Plasma lipid profile

Effect of BM on liver antioxidants profile

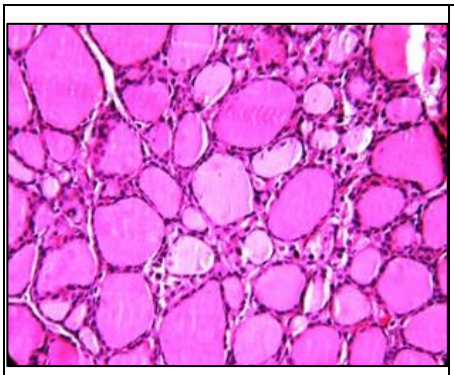
Table 3 shows the levels of hepatic antioxidants in control and experimental rats. Significant increase in the level of LPO with the concomitant decrease in CAT, SOD and GSH content were observed in hypothyroid group when compared to control rats. Supplementation with BM caused significant restoration in the levels of CAT, SOD and GSH and LPO products when compared to hypothyroid group.

Table 3: Effect of BM on liver antioxidants profile and lipid peroxidation

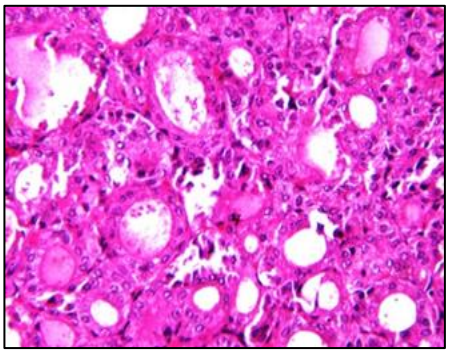
Parameters	Group I	Group II	Group III	Group IV	Group V
SOD* (U/mg protein)	$18.65^{b} \pm 0.77$	$12.27^{c} \pm 0.36$	$18.25^{b} \pm 0.42$	$20.72^{b} \pm 0.68$	$22.54^{a} \pm 0.65$
CAT* (U/mg protein)	$20.60^{b} \pm 0.54$	$15.62^{c} \pm 0.61$	$20.72^{b} \pm 0.43$	$21.32^{b} \pm 0.65$	$23.76^{a} \pm 0.35$
GSH*(nmole/mg protein)	$23.62^{a} \pm 0.74$	$12.36^{b} \pm 0.61$	$21.47^{a} \pm 0.65$	$23.68^{a} \pm 0.57$	$23.77^{a} \pm 0.67$
MDA*(nmole/g tissue)	$1.66^{b} \pm 0.53$	$3.08^{a} \pm 0.62$	$1.68^{b} \pm 0.42$	$1.48^{b} \pm 0.54$	$1.04^{bc} \pm 0.61$

*Overall means bearing different superscripts within row differ highly significantly (P<0.01) Values were expressed as mean ± SEM for 6 rats in each group

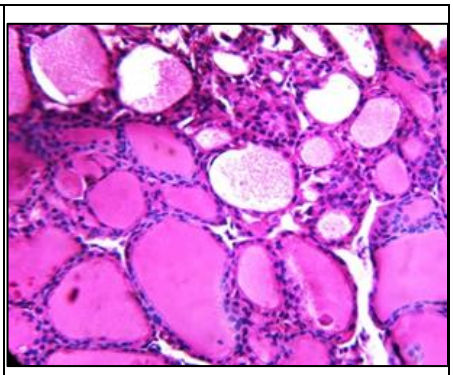
Effect of BM on thyroid morphology



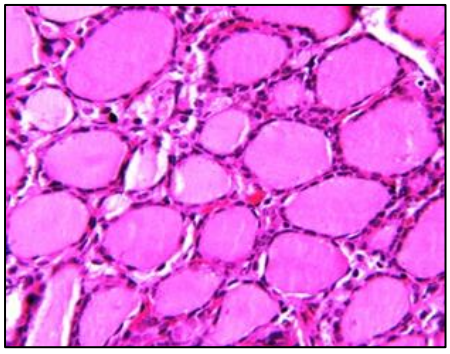
A: Normal architecture of thyroid follicles (Group I)



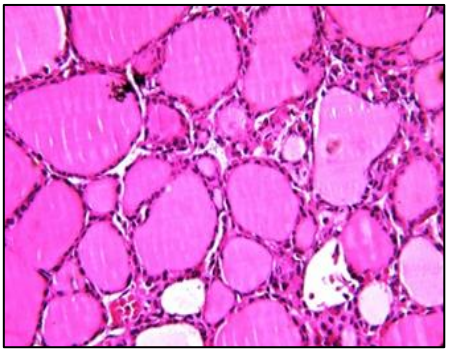
2B: Degeneration and necrosis of thyroid follicles (Group II)



2C: Mixed follicles of various sizes (Group II)



2D: Normal sized follicles completely filled with colloid (Group IV)



2E: Normal thyroid follicles with abundant colloid (Group V)

Fig 2

Figure 2(A-E) portraits the effect of BM on thyroid morphology in experimental rats. Thyroid gland of control group showed thyroid follicles of normal size completely filled with colloid and well differentiated stroma. Thyroid gland from hypothyroid group showed loss of follicular architecture, indicating degeneration and necrosis of follicular epithelial and stromal cells with scanty colloid. Thyroid gland from levothyroxine treated group showed mixed follicles of various sizes with incompletely filled colloid and moderate mononuclear cellular infiltration in the stroma. Thyroid glands from BM 200 mg treated group showed normal sized follicles completely filled with colloid and minimal mononuclear cellular infiltration in the stroma. Herbal control group showed thyroid follicles of normal size with abundant colloid indicating the thyroid stimulatory action of the herb.

Discussion

In the present study, the pharmacological effect of *B. monnieri* on experimental hypothyroidism and associated complications were assessed. *B. monnieri* administration at 200 mg/kg b.wt in hypothyroid rats (group IV), reversed the plasma levels of T_3 and T_4 to normal range on par with standard drug levothyroxine and TSH level was reduced because of servo mechanism. In euthyroid rats (group V), *B. monnieri* administration increased the production of T_4 and T_3, but the level of increment of T_3 is lesser than T_4. Kar and Panda, reported that administration of ethanolic extract of *B. monnieri* increased only T_4 and not T_3 in euthyroid mice and the plant extract might be stimulating the synthesis and/or release of T_4 directly at the glandular level but not through the peripheral conversion of T_4 to T_3 [9]. Estimation of TSH was not carried out in that study. In our study, *B. monnieri* increased both T_4 and T_3 in hypothyroid and euthyroid animals and TSH level was reduced, which revealed that thyrogenic action of BM is not only localized in the thyroid gland but the herb might have some central or peripheral actions.

BM treated groups showed significant increase in liver antioxidant enzymes such as CAT, SOD, GSH and decrease in lipid peroxidation (LPO). It has been already reported that BM has an antioxidant activity owing to the presence of its saponins, flavonoids and phytosterol, thus it reduces the concentration of free radicals, which might prevent the initiation and propagation of LPO [16].

Deficiency of thyroid hormone decreases HMG CoA reductase, which is a rate controlling enzyme in metabolic pathway of lipids, down regulates LDL receptors, impairs sterol regulator element binding protein 2 (SREBP-2), which is responsible for increase in total cholesterol, LDL and VLDL cholesterol. Whereas down regulation of apolipoprotein AV due to thyroid hormone deficiency increases triglycerides [17]. BM treated groups showed significant reduction in plasma lipid profile when compared to hypothyroid group. Kamesh and Sumathy studied the antihypercholesterolemic effect of BM on high cholesterol diet induced hypercholesterolemia in rats and found out that BM treatment significantly reduced the levels of TC, LDL, VLDL, TG and increased the level of HDL when compared to hypercholesterolemic rats [18]. The hypolipidemic activity of BM might be due to its phytocompounds such as saponins and flavonoids.

Saponins are reported to lower plasma cholesterol level by interfering with intestinal absorption of cholesterol; it also lowers plasma triglycerides by inhibiting the pancreatic enzyme, lipoprotein lipase. Flavonoids are reported to increase HDL-C and decrease oxidation of LDL-cholesterol

[19]. Similarly, in this study also, the presence of both saponins and flavonoids in BM could have contributed in lowering the lipid profile. So far no results have been reported on the effect of BM on lipid profile in hypothyroidism.

Histopathology of hypothyroid group revealed altered architecture of thyroid follicles, degeneration and necrosis of follicular epithelium and stromal cells with scanty colloid. The result was in accordance with Zaidi *et al*. [20]. BM treated groups showed normal architecture of thyroid follicles completely filled with colloid and minimal mononuclear infiltration, indicating the thyroid regenerative potential of BM in hypothyroid rats.

Conclusion

The result obtained in this study suggests that the alcoholic extract of *B. monnieri* has beneficial effects in preventing hypothyroidism and complications associated with it, by increasing thyroid hormones production from the thyroid gland, improving antioxidant status, lowering lipid profile, as well as protecting the thyroid morphology.

References

1. Bello F, Bakari AG. Hypothyroidism in adults: A review and recent advances in management. J Diabetes Endocrinol 2012;3(5):57-69.
2. Srikala S, Sathishumar. Hypothyroidism associated systemic and peripheral disorders in dogs. Animal science reporter 2014;8(1):31-40.
3. Alkalby JMA, Alzerjawi SJS. Effect of propylthiouracil-induced hypothyroidism on reproductive efficiency of adult male rats. Bas. J Vet. Res 2013;12(2):113- 21.
4. Katzung BG, Trever AJ. Basic and Clinical Pharmacology. Edn 13, McGraw Hill Publishers, New York 2015; 1:920-34.
5. Rai D, Bhatia G, Palit G, Pal R, Singh S, Singh HK, *et al*. Adaptogenic effect of *Bacopa monniera* (Brahmi). Pharmacol. Biochem. Behav 2003;75(4):823-30.
6. Russo A, Izzo AA, Borrelli F, Renis M, Vanella A. Free radical scavenging capacity and protective effect of *Bacopa monniera* L. on DNA damage. Phytotherapy Research 2003;17(8):870-75.
7. Channa S, Dar A, Anjum S, Yaqoob M, Atta-Ur-Rahman: Anti-inflammatory activity of *Bacopa monnieri* in rodents. J Ethnopharmacol 2006;104(1-2):286-89.
8. Lal S, Baraik B. Phytochemical and pharmacological profile of *Bacopa monnieri* - an ethnomedicinal plant. Int J Pharm Sci & Res 2019;10(3):1001-13.
9. Kar A, Panda S, Bharti S. Relative efficacy of three medicinal plant extracts in the alteration of thyroid hormone concentrations in male mice. J Ethnopharmacol 2002;81(2):281-85.
10. Blessy B Mathew, Suresh K Jatawa, Archana Tiwari. Phytochemical Analysis of Citrus Limonum Pulp and peel. Int J Pharmacy Pharm Sci 2012;4(2):269-371.
11. Friedewald J, Levy YR, Friedrickson SD. Estimation of concentration of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. Clin. Chem 1972;18(6):499-502.
12. Marklund SL, Marklund G. Involvement of superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J Biochem 1974;47(3):469-74.
13. Aebi H. Catalase *in vitro*. Method Enzymol 1984;105:121-26.
14. Moron MJ, Depiere JW, Mannirvik B. Levels of glutathione, glutathione reductase and glutathione S transferase activities in rat lung and liver. Biochem. Biophys. Acta 1979;582(1):67-78.
15. Okawa H. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem 1979;95(2):351-58.
16. Tripathi YB, Chaurasia S, Tripathi E, Upadhyay A, Dubey GP. *Bacopa monniera* Linn. As an antioxidant: mechanism of action. Indian J Exp Biol 1996;34(6):523-526.
17. Rizos CV, Elisaf MS, Liberopoulos EN. Effects of thyroid dysfunction on lipid profile. Open Cardiovasc. Med. J 2011;5:76-84.
18. Kamesh V, Sumathy T. Anti hypercholesterolemic effect of *Bacopa monniera* Linn. on high cholesterol diet induced hypercholesterolemia in rats. Asian Pac. J Trop Med 2012;5(12):949-55.
19. Li H, Wang QJ, Zhu DN, Yang Y, Reinioside C. A triterpene saponin of *Polygala aureocauda* Dunn, exerts hypolipidemic effect on hyperlipidemic mice. Phytother Res 2008;22(2):159-64.
20. Zaidi TM, Khan AA, Hasan BM, Faruqi AN. Carbimazole induced thyroid histopathy in albino rats during development. J Anat Soc India 2004;53(2):14-17.